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LIGHT AND FINE STRUCTURAL STUDIES OF NATURAL AND ARTIFICIALLY INDUCED EGG GROWTH OF PENAEID SHRIMP[1]

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ABSTRACT

Egg differentiation in penaeids induced to undergo gonad maturation by eyestalk ablation has been examined with light and electron microscopes.

Controls consisted of both wild brown and white shrimp (Penaeus aztecus and P. setiferus) caught in various stages of ovarian maturation. These animals were sacrificed and ovarian tissues were excised and fixed in a cold (4 C) aldehyde mixture, post-fixed in osmic acid, dehydrated in acetone, and embedded in an epoxy resin. Experimental animals included laboratory-reared, wild immature, and wild mature animals which had resorbed ovarian tissues. These animals were ablated with hot surgical clamps and sacrificed at various time intervals. Tissues were prepared as described for the controls.

The development of ova from experimental animals was compared to control animals. In the stages early oocyte, cisternal phase, platelet phase, cortical rod phase, and mature oocyte, the experimental oocytes appeared the same as the control tissues. Though further studies (e.g., biochemical and zygote development) are needed, these preliminary fine structural studies are encouraging. While ablation, a mechanical process, may not be the technique of choice for maturing shrimp, it does indicate that manipulation of the endocrine system will be successful.

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INTRODUCTION

Our laboratory as well as several other groups have noted fortuitous maturation and spawning of penaeids held in captivity (Michel, personal communication, and Moore and Sherry, 1974). Unfortunately these events have been at the whim of the animals involved and thus have been unpredictable. Laboratories or businesses dealing in the future with breeding stocks will not be able to cope with such happenstance events but, rather, will need reliable and thoroughly predictable techniques at their disposal.

In the past, several investigators have indicated that eyestalk ablation results in gonad maturation among members of the decapod Crustacea (Adiyodi and Adiyodi, 1970). Unfortunately, reports dealing with the penaeids (Idyll, 1971; Caillouet, 1973) did not indicate whether the germ cells from ablated animals were viable. We have initiated studies to determine the viability of eggs produced by ablated penaeid shrimp. This report deals with the fine structure studies thus far completed.

MATERIALS AND METHODS

Brown shrimp (Penaeus aztecus) were taken with an otter trawl on the shrimp fishing grounds (73-92 m) south of Freeport, Texas. White shrimp (P. setiferus) were collected from tidal passes and shallow (4-9 m) Gulf waters in the Galveston, Texas, area. Hatchery-reared P. aztecus (mean total length, 136 mm) were used in one experiment. All animals were maintained in aerated tanks equipped with sand-shell substrates and were fed an extruded pellet developed in cooperation with a commercial feed producer.

Bilateral eyestalk ablation was employed to stimulate egg growth and maturation in hatchery-reared, wild immature, and wild shrimp with reabsorbed eggs. Ablations were accomplished by cauterization with hot surgical clamps to minimize the loss of body fluids. The antibiotic, Aureomycin (use of trade names does not imply endorsement of commercial products), was applied to cauterized areas to help prevent infection.

Control animals, in various stages of ovarian development, were sacrificed upon capture and ovarian tissues were removed. Ablated animals were sacrificed at 24-hour intervals, and ovarian tissues were removed. Excised tissue was immediately immersed in a paraformaldehyde-glutaraldehyde fixative (Karnovsky, 1965) at room temperature. The dissected ovary was then minced and placed in fresh fixative for 1-2 hours. The tissue was then post-fixed in 1.0% osmium tetroxide buffered with 0.1 M phosphate (Millonig, 1961) for one hour at 4 C. The tissue was dehydrated in a graded acetone series, embedded in a low viscosity epoxy resin (Spurr, 1969), and sectioned on a Sorval M-T2B ultramicrotome with glass or diamond knives. Thin sections were mounted on uncoated copper grids, stained with methanolic uranyl acetate and lead citrate, and examined with an Hitachi HS-8 electron

microscope. Thick sections, 0.5-1.0 μ, for light microscopy were stained with 1.0% aqueous methelene blue in 1.0% sodium borate.

RESULTS

Control Oocyte Growth

To facilitate an understanding of ovarian maturation, we have divided egg development into five main stages (early oocyte, cisternal phase, platelet phase, cortical rod phase, and mature oocyte) which can be easily determined at the light microscopic level. Other investigators have proposed various schemes for the staging of penaeid oocyte growth (Hudinaga, 1942; King, 1948; Oka and Shirahata, 1965; Burukovskii, 1970). Our stages differ from those proposed in the past. This difference does not represent disagreement in results but, rather, reflects the use of advanced techniques.

Early Oocyte

The early oocytes are round to oval cells, approximately 65 μ in diameter, containing large, centrally located nuclei. The nuclei contain several large circular nucleoli in the peripheral karyoplasm (Figure 1).

At the fine structural level, the cytoplasm of these cells is found to contain numerous ribosomes and a few mitochondria. The perinuclear cytoplasm contains large aggregates of electron dense material that are closely associated with nuclear pores (Figures 2-4).

This stage is found in the ovaries of immature shrimp as small as 50 mm in length. The first stages of sexual maturation or oocyte development are evidenced by yolk deposition as demonstrated in the cisternal phase.

Cisternal Phase

At the light microscopic level, this stage is recognized by the appearance of light staining patches uniformly distributed throughout the cytoplasm. Also, dark staining cytoplasmic bodies are apparent around the nuclear envelope and in the perinuclear cytoplasm. It is during this stage that folicle cells begin to surround the oocyte (Figure 5).

Extensive perinuclear activity can be seen in this phase (Figures 6 and 7). Aggregates of electron dense material are apparent around the nuclear envelope. The cytoplasm contains more mitochondria than in the previous stage and Golgi bodies and endoplasmic reticulum make a conspicuous appearance. The reticular elements,

which arise from the outer component of the nuclear envelope (Figure 7), become dilated with electron dense material. Eventually these cells become engorged with the swollen reticular elements which are believed to be intraoocytic protein yolk (Figure 8).

Platelet Phase

During this phase, the oocyte greatly increases in size (250 μ), is surrounded by a distinct layer of follicular cells, and contains dense yolk platelets (Figure 9).

At the fine structural level, the formation of these platelets can be defined. Numerous micropinocytotic vesicles, which originate from the plasma membrane of the oocyte, move into the cytoplasm and fuse with each other, giving rise to the yolk spheres (Figure 10). The spheres or platelets (Figure 11) are probably composed of a lipoprotein from an extraoocytic source. They are distinct from the swollen reticular elements containing intraoocytic protein yolk.

Cortical Rod Phase

During this stage of development, the oocytes contain dense accumulations of lipid yolk and are surrounded by attenuated follicle cells. The most characteristic features of this stage, however, are large (2 to 9 μ) inclusions (cortical rods) found in the peripheral ooplasm (Figure 12).

At the ultrastructural level these cortical rods exhibit a distinct substructure (Figure 13). The matrix of these rods is packed with small "feathery" structures (Figure 14). Each of these structures has an electron dense axis 0.36 μ long and 24 mμ in diameter. Projecting from this axis are electron dense fibrillar elements 48 mμ in diameter. Projecting from this axis are electron dense fibrillar elements 48 mμ long and 4 mμ in diameter.

Mature Oocyte

The mature oocyte is similar to the cortical rod phase with one striking exception. Shortly before ovulation the cortical rods move to the periphery of the oocyte and dehisce (Figures 15 and 16).

This work has shown very interesting features concerning oocyte growth in the penaeids. Of the arthropods studied to date, three distinct types of yolk production or accumulation have been described. The insects exhibit extra-oocytic production of yolk with the subsequent uptake of this material by the oocyte (Cone and Scalizi, 1967); the crustaceans demonstrate intra-oocyte production of yolk (for review see Kessel, 1968; and Hinsch and Cone, 1969); and the xyphosurans utilize both intra-oocytic yolk production and the uptake of preformed yolk from the hemolymph (Dumont and Anderson, 1967).

The penaeids do not demonstrate the "typical" pattern found in other crustaceans. Instead they exhibit growth patterns more common to the xyphosurans. Another very interesting feature of penaeid oocytes are the striking cortical specializations (rods) which become apparent late in the growth of the oocyte. To our knowledge cortical specializations of a similar nature have not been described in the eggs of any other animals.

Experimental Oocyte Growth

A total of five ablation experiments are reported in this paper and outlined in Table 1. In all instances where egg growth was noted in ablated animals, the developing oocytes appeared normal and similar to the control group at the microscopic level (only the control set of figures is presented). However, in no instance did an animal with fully developed oocytes spawn. Instead, the ovaries, after reaching full growth, regressed and exhibited reabsorption of all mature oocytes.

During ablation experiments water temperature, pH, and salinity were controlled. As these parameters were altered there appeared to be a distinct variation in maturation time. Unfortunately, these were preliminary experiments and discussion concerning the effects of individual parameters is not possible at this time. Continuing experiments are in progress to determine the effect of each environmental condition. It is felt this is critical since the time span of oocyte growth may be very significant, especially when one considers the various processes taking place during this growth period. Not only are yolk and cortical specializations produced and organized during this time, but informasomal materials (ribonucleoproteins, and ribonucleic acids) necessary for early zygote development are also produced. If any one of the above processes is changed due to alterations in time, the success of early development would be drastically affected.

Ablation experiments have also dramatized the distinction between oocyte growth and maturation. While oocytes from ablated animals demonstrated normal growth, they do not exhibit maturation; that is, they do not undergo meiosis (unpublished data). These results suggest that while growth is under "Y" gland control, maturation may not be.

SUMMARY

This work represents initial attempts to determine the viability of eggs produced by ablated females. While this work is by no means complete, we have presented data pertinent to the ablation technique and oogenesis in general. 1) A series of oocyte growth stages directly related to metabolic transitions are described at the microscopic level. 2) Penaeid oocytes apparently accumulate two distinct types of yolk, one produced by the oocyte and a second pinocytotically removed from the hemolymph. 3) Oocytes of ablated animals exhibit normal growth, but not maturation. 4) Environmental parameters (temperature, pH, and salinity), either by themselves or in conjunction with each other, apparently influence oocyte growth rate in ablated females.

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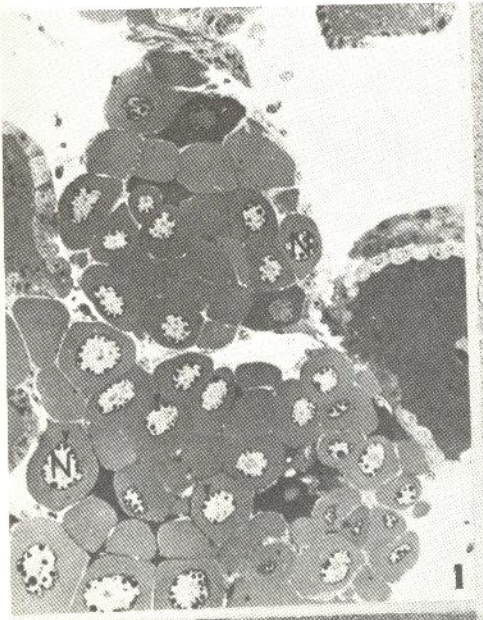
Table 1. - Summary of ablation results

Source of animals	Environmental Parameters: Temperature C	pH	Salinity ppt	Number of animals	Mortalities	Results* (observations)	Days to attain complete growth
Penaeus aztecus (wild immature)	17	7.6	28	10	0	Complete growth (2) Partial growth (6) No development (2)	10
P. aztecus (hatchery reared)	20	7.6	30	14	0	Complete growth (6) Partial growth (8)	11
P. aztecus (resorbed)	22	--	35	30	6	Complete growth (11) Partial growth (10) No development (3)	7
P. aztecus (wild immature)	27	8.7	34	32	8	Complete growth (14) Partial growth (10)	5
P. setiferus (wild immature)	27	8.0	34	42	14	Complete growth (8) Partial growth (11) No development (9)	4

* Complete growth: Indicates mature oocyte.

Partial growth: Means oocyte growth was arrested without explanation or female with developing oocytes was sacrificed.

No development: Oocyte growth was not observed.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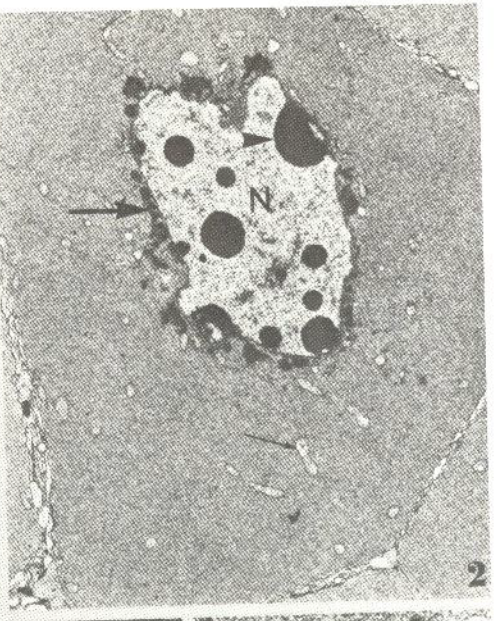
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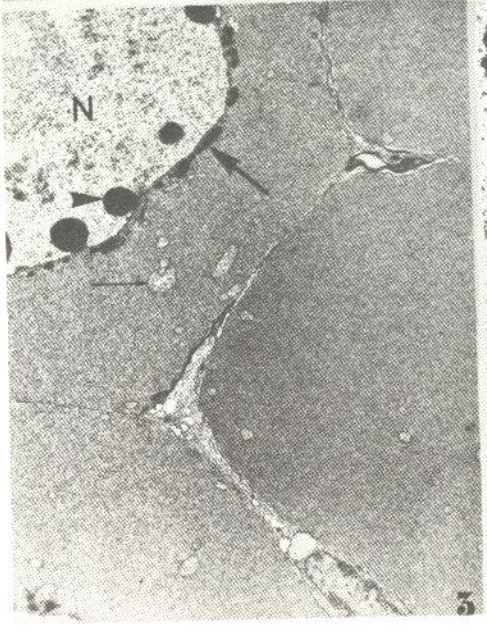
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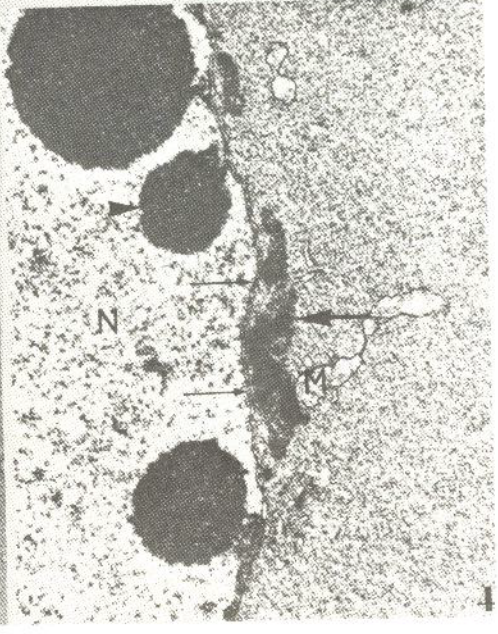
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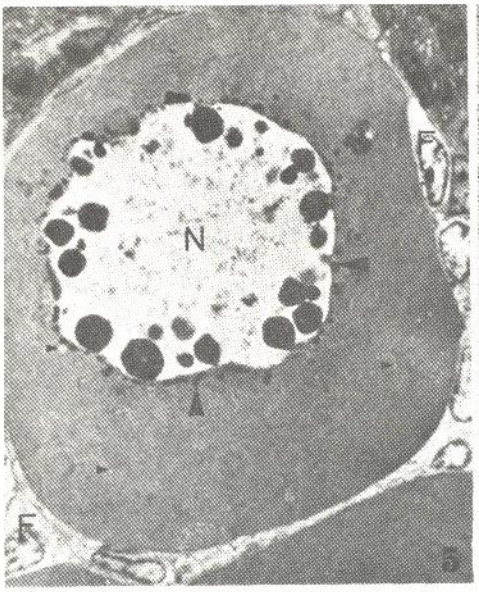
Figure 1. A light micrograph showing several early oocytes with centrally located nuclei (N). X800

Figure 2. and Figure 3. Electron micrographs of early oocytes. These cells contain centrally located nuclei (N) which contain several nucleoli (large arrowhead). The ooplasm contains a few mitochondria (small arrows) and dense material around the nuclear envelope (large arrows). X2,100 and X4,400

Figure 4. An electron micrograph showing a portion of an oocyte nucleus (N), nucleoli (large arrowhead), nuclear pores (small arrows), dense ooplasmic material associated with the pores (large arrow), and mitochondria (M). X12,000



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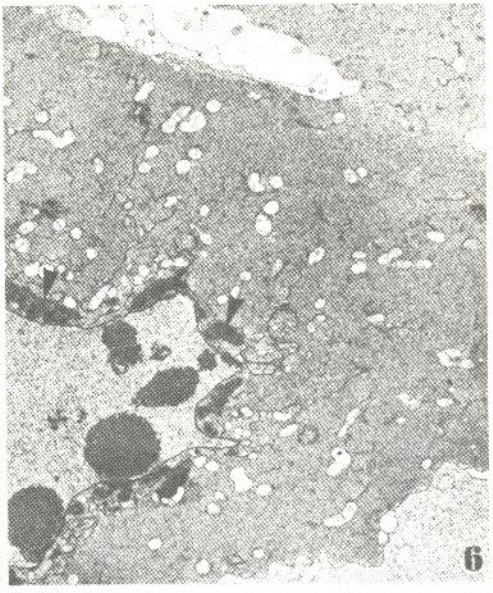

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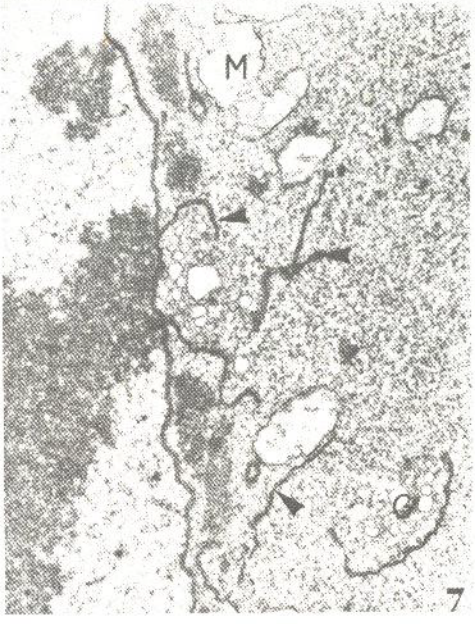

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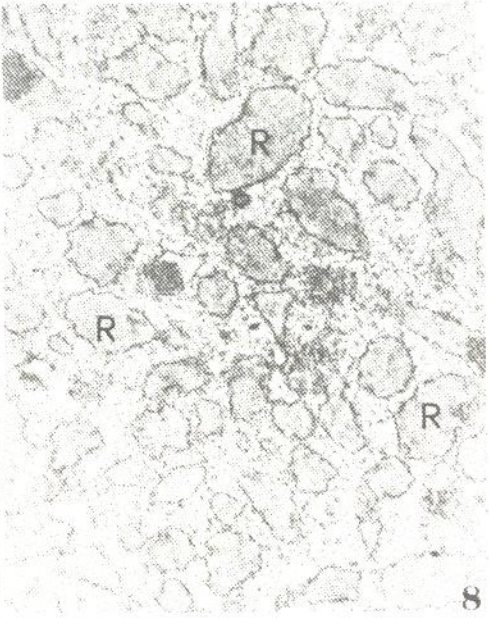

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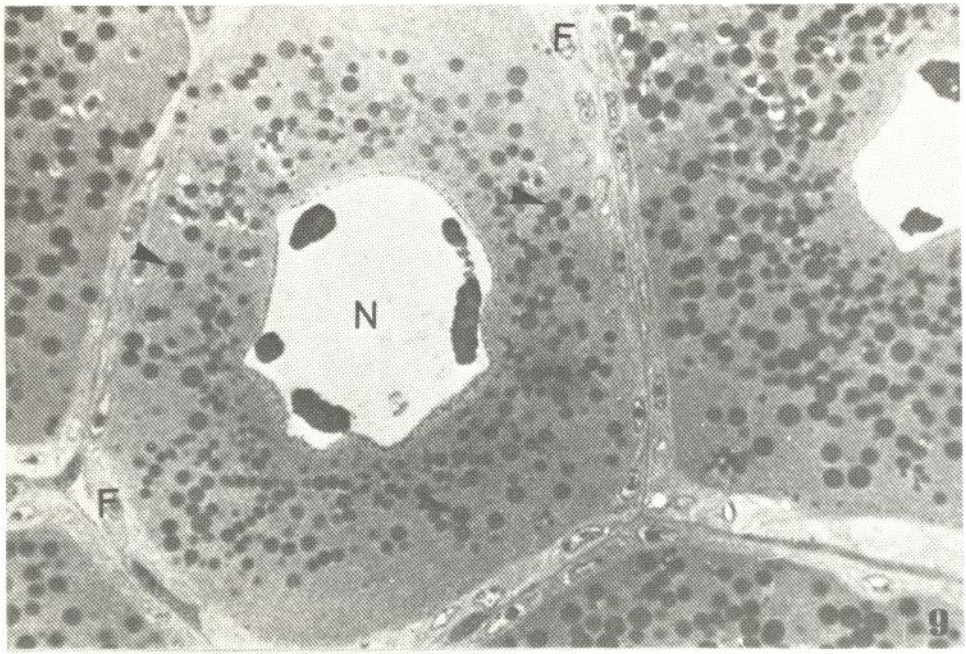
Figure 5. A light micrograph of the cisternal phase oocyte. These cells contain centrally located nuclei (N). Dark staining bodies are present in the perinuclear cytoplasm (large arrowheads); light staining areas are found throughout the cytoplasm (small arrowheads); and follicle cells (F) are present. X2,000

Figure 6. Electron micrograph of cisternal phase oocyte showing electron dense material in perinuclear cytoplasm (large arrowheads). X6,100

Figure 7. High magnification of portion of a cisternal phase oocyte showing reticular elements (large arrowheads), mitichondria (M) and Golgi bodies (G). X19,000

Figure 8. Electron micrograph of reticular elements (R) swollen with protein yolk. X19,000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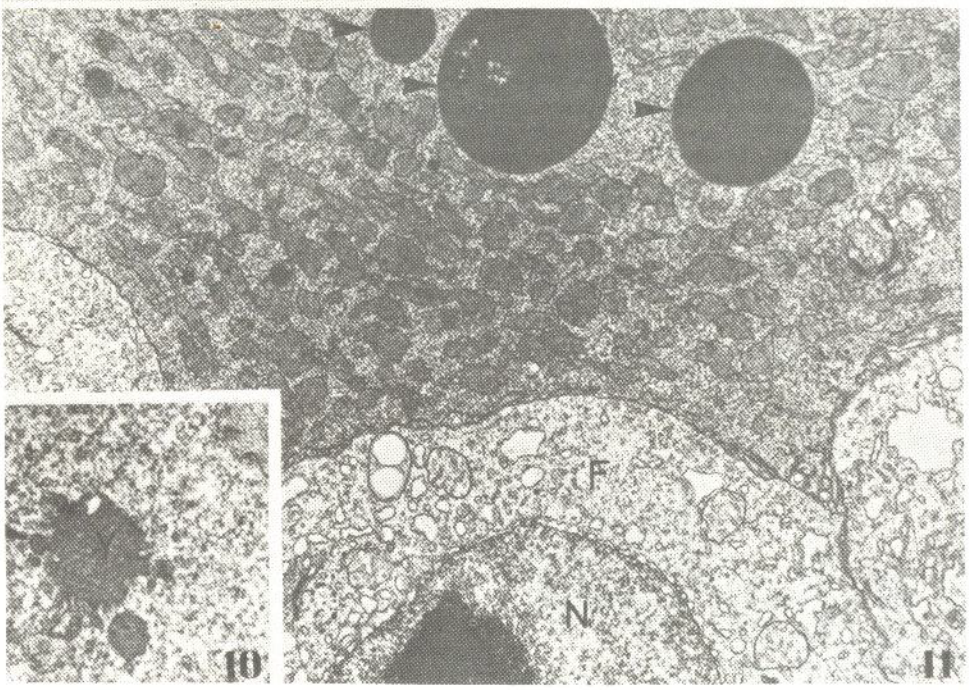
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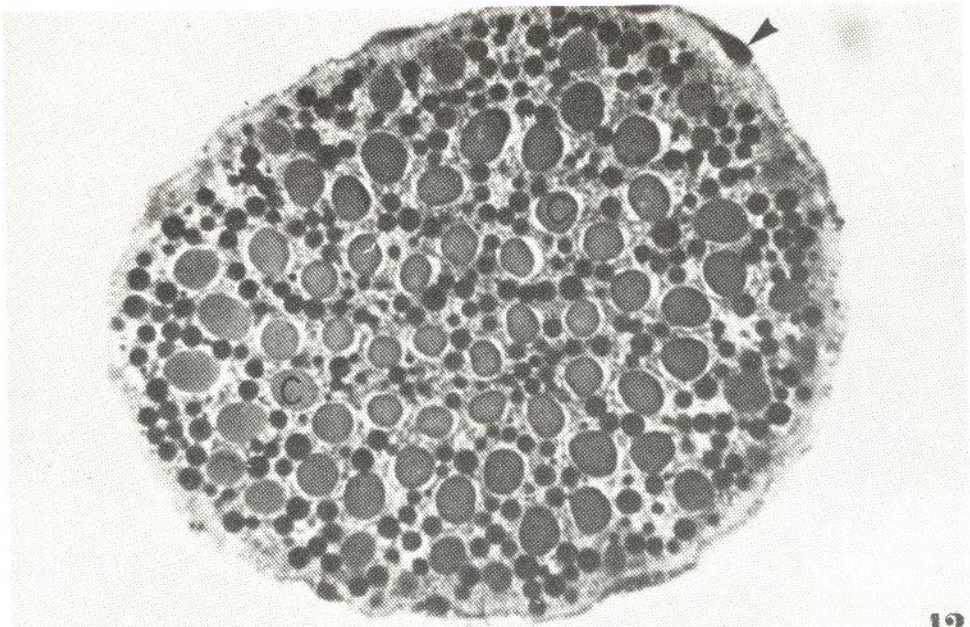
Figure 9. Light micrograph of platelet phase oocyte. This cell has a large nucleus (N), lipid yolk platelets (large arrowheads), and is surrounded by follicle cells (F). X1,600

Figure 10. Electron micrograph showing formation of yolk platelet (Y) by the fusion of micropinocytotic vesicles (large arrowhead). X19,000

Figure 11. Electron micrograph of peripheral portion of platelet phase oocyte containing yolk spheres (large arrowheads). Follicle cells (F) and a follicle cell nucleus (N) are shown. X4,400



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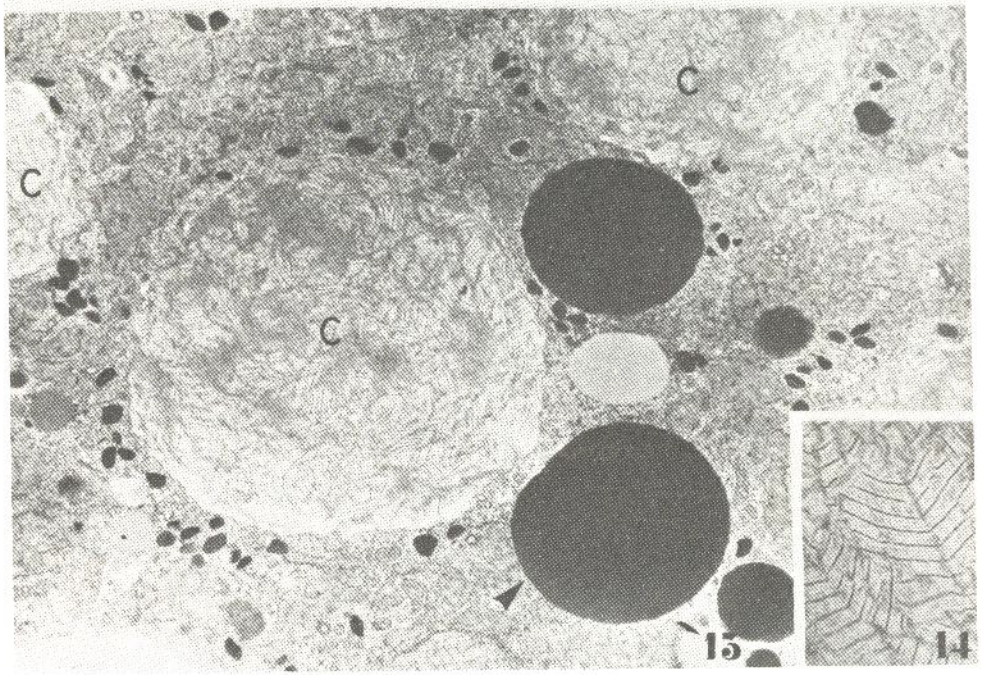

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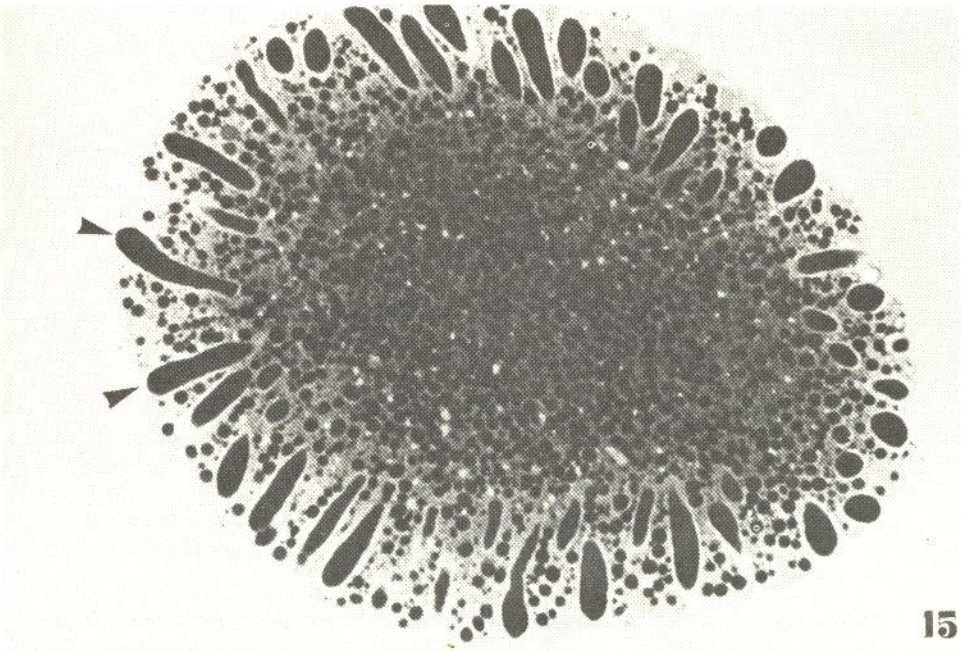
Figure 12. Light micrograph of oocyte containing numerous developing cortical rods (C). This cell is surrounded by follicle cells as evidenced by their nuclei (large arrowhead). X900

Figure 13. Electron micrograph of cortical rod phase oocyte, showing cortical rods (C) and lipid yolk platelets (large arrowhead). X6,100

Figure 14. Higher magnification of cortical rod showing details of "feathery" matrix. X19,000



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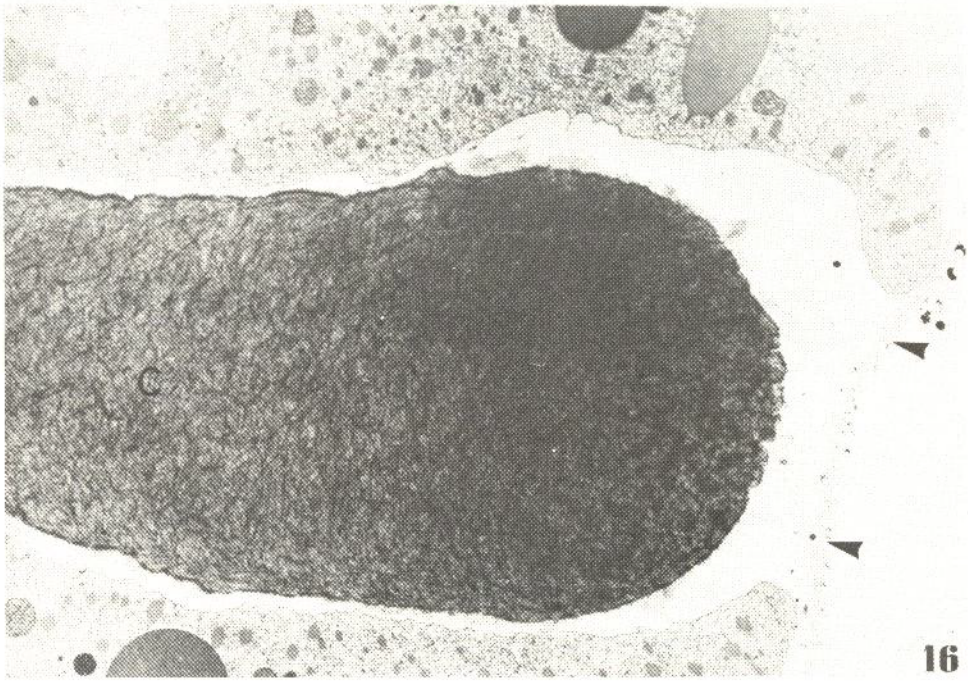

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Figure 15. Light micrograph of mature oocyte showing dense accumulation of yolk and peripheral cortical rods (large arrowheads). X800

Figure 16. Electron micrograph of mature oocyte showing open cortical rod (C). An investing coat (large arrowheads) surrounds oocyte. X2,100